

**Amendments to the Specification:**

Please replace the paragraph on page 11, line 26, with the following amended paragraph:

Plasmid phuOPGbp 1.1 in E. coli strain DH10 encoding human OPG binding protein was deposited with the American Type Culture Collection, ~~Rockville, MD~~ 10801 University Boulevard, Manassas, VA 20110-2209 on June 13, 1997.

Please replace the paragraph beginning on page 50, line 27, with the following amended paragraph:

Concurrently, log phase cultures of myeloma cells, Sp2/0-Ag14 or Y3-Ag1.2.3 for mouse or rat splenocyte fusions, respectively, (American Type Culture Collection; ~~Rockville, MD~~ 10801 University Boulevard, Manassas, VA 20110-2209) grown in complete medium (DMEM, 10% inactivated fetal bovine serum, 2 mM glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, and 10 mM hepes buffer; Gibco Laboratories, Grand Island, NY) are washed in similar fashion. The splenocytes are combined with the myeloma cells and pelleted once again. The media is aspirated from the cell pellet and 2 ml of polyethylene glycol 1500 (PEG 1500; Boehringer Mannheim Biochemicals, Indianapolis, IN) is gently mixed into the cells over the course of 1 minute. Thereafter, an equal volume of 2x P/S/G DMEM is slowly added. The cells are allowed to fuse at 37° C for 2 minutes, then an additional 6 ml of 2x P/S/G DMEM is added. The cells are again set at 37°C for 3 minutes. Finally, 35 ml of 2x P/S/G DMEM is added to the cell suspension, and the cells pelleted by centrifugation. Media is aspirated from the pellet and the cells gently resuspended in complete medium. The cells are distributed over 96-well flat-bottom tissue culture plates (Becton Dickinson Labware; Lincoln Park, NJ) by single drops from a 5 ml pipette. Plates are incubated overnight in humidified

conditions at 37°C , 5% CO<sub>2</sub>. The next day, an equal volume of selection medium is added to each well. Selection consists of 0.1 mM hypoxanthine, 4 x 10<sup>-4</sup> mM aminopterin, and 1.6 x 10<sup>-2</sup> mM thymidine in complete medium. The fusion plates are incubated for 7 days followed by 2 changes of medium during the next 3 days; HAT selection medium is used after each fluid change. Tissue culture supernatants are taken 3 to 4 days after the last fluid change from each hybrid-containing well and tested by EIA for specific antibody reactivity. This protocol has been modified by that in Hudson and Hay, "Practical Immunology, Second Edition", Blackwell Scientific Publications.

Please replace the paragraph beginning on page 24, line 7, with the following amended paragraph:

Cell lines that grew as adherent cultures in vitro were treated using the following methods: Cells were plated into 24 well tissue culture plates (Falcon), then allowed to grow to approximately 80% confluency. The growth media was then removed, and the adherent cultures were washed with phosphate buffered saline (PBS) (Gibco) containing 1% fetal calf serum (FCS). Recombinant mouse OPG [22-194]-Fc and human OPG [22-201]-Fc fusion proteins (see U.S. Serial No. 08/706,945 filed September 3, 1996 now U.S. Patent No. 6,369,027) were individually diluted to 5 ug/ml in PBS containing 1% FCS, then added to the cultures and allowed to incubate for 45 min at 0°C. The OPG-Fc fusion protein solution was discarded, and the cells were washed in PBS-FCS solution as described above. The cultures were then exposed to phycoerythrin-conjugated goat F(ab') anti-human IgG secondary antibody (Southern Biotechnology Associates Cat. # 2043-09) diluted into PBS-FCS. After a 30-45 min incubation at 0°C, the solution was discarded, and the cultures were washed as described above. The cells were then analysed by immunofluorescent microscopy to detect cell lines which express a cell surface OPG binding protein.

Please replace the paragraph beginning on page 38, line 27, with the following amended paragraph:

Recombinant bacterial constructs pAMG21-murine OPG binding protein [75-316], [95-316], [107-316], [118-316], [128-316], [137-316], and [158-316] have been cloned, DNA sequence confirmed, and levels of recombinant gene product expression following induction has been examined. All constructs produced levels of recombinant gene product which was readily visible following SDS polyacrylamide gel electrophoresis and coomassie staining of crude lysates. Growth of transformed E. coli 393 or 2596, induction of OPG binding protein expression and isolation of inclusion bodies containing OPG binding protein is done according to procedures described in U.S. Serial No. 08/577,788 filed December 22, 1995 now U.S. Patent No. 6,613,544. Purification of OPG binding proteins from inclusion bodies requires solubilization and renaturing of OPG binding protein using procedures available to one skilled in the art. Recombinant murine OPG binding protein [158-316] was found to be produced mostly insolubly, but about 40% was found in the soluble fraction. Recombinant protein was purified from the soluble fraction as described below and its bioactivity examined.

Please replace the paragraph beginning on page 40, line 10, with the following amended paragraph:

Recombinant OPG protein has previously been shown to block vitamin D3-dependent osteoclast formation from bone marrow and spleen precursors in an osteoclast forming assay as described in U.S. Serial No. 08/577,788 now U.S. Patent No. 6,613,544. Since OPG binding protein binds to OPG, and is a novel member of the TNF family of ligands, it is a potential target of OPG bioactivity. Recombinant soluble OPG binding protein (158-316), representing the minimal core TNF $\alpha$ -like domain, was tested for its ability to modulate osteoclast differentiation from

osteoclast precursors. Bone marrow cells were isolated from adult mouse femurs, and treated with M-CSF. The non-adherent fraction was co-cultured with ST2 cells in the presence and absence of both vitamin D3 and dexamethasone. As previously shown, osteoclasts develop only from co-cultures containing stromal cells (ST2), vitamin D3 and dexamethasone. Recombinant soluble OPG binding protein was added at varying concentrations ranging from 0.16 to 500 ng/ml and osteoclast maturation was determined by TRAP solution assay and by visual observation. OPG binding protein strongly stimulated osteoclast differentiation and maturation in a dose dependent manner, with half-maximal effects in the 1-2 ng/ml range, suggesting that it acts as a potent inducer of osteoclastogenesis in vitro (Figure 5). The effect of OPG binding protein is blocked by recombinant OPG (Figure 6).

Please replace the paragraph beginning on page 43, line 10, with the following amended paragraph:

The full length clone of murine and human OPG binding protein can be expressed in mammalian cells as previously described in Example 2. Alternatively, the cDNA clones can be modified to encode secreted forms of the protein when expressed in mammalian cells. To do this, the natural 5' end of the cDNA encoding the initiation codon, and extending approximately through the first 69 amino acid of the protein, including the transmembrane spanning region, could be replaced with a signal peptide leader sequence. For example, DNA sequences encoding the initiation codon and signal peptide of a known gene can be spliced to the OPG binding protein cDNA sequence beginning anywhere after the region encoding amino acid residue 68. The resulting recombinant clones are predicted to produce secreted forms of OPG binding protein in mammalian cells, and should undergo post translational modifications which normally occur in the C-terminal extracellular domain of OPG binding protein, such as glycosylation. Using this strategy, a secreted form of OPG

binding protein was constructed which has at its 5' end the murine OPG signal peptide, and at its 3' end the human IgG1 Fc domain. The plasmid vector pCEP4/muOPG[22-401]-Fc as described in U.S. Serial No. 08/577,788, filed December 22, 1995, now U.S. Patent No. 6,613,544, was digested with NotI to cleave between the 3' end of OPG and the Fc gene. The linearized DNA was then partially digested with XmnI to cleave only between residues 23 and 24 of OPG leaving a blunt end. The restriction digests were then dephosphorylated with CIP and the vector portion of this digest (including residues 1-23 of OPG and Fc) was gel purified.

Please replace the paragraph beginning on page 44, line 13, with the following amended paragraph:

The 1602-61 oligonucleotide amplifies the 5' end of the gene and contains an artificial an StuI site. The 1602-59 primer amplifies the 3' end of the gene and contains an artificial NotI site. The resulting PCR product obtained was digested with NotI and StuI, then gel purified. The purified PCR product was ligated with vector, then used to transform electrocompetent E. coli DH10B cells. The resulting clone was sequenced to confirm the integrity of the amplified sequence and restriction site junctions. This plasmid was then used to transfect human 293 fibroblasts, and the OPG binding protein-Fc fusion protein was collected from culture media as previously described in U.S. Serial No. 08/577,788, filed December 22, 1995 now U.S. Patent No. 6,613,544.

Please replace the paragraph beginning on page 44, line 27, with the following amended paragraph:

Using a similar strategy, an expression vector was designed that is capable of expressing a N-terminal truncation of fused to the human IgG1 Fc domain. This construct consists of the murine OPG signal peptide (aa residue 1-21), fused in frame to murine

OPG binding protein residues 158-316, followed by an inframe fusion to human IgG1 Fc domain. To do this, the plasmid vector pCEP4/ murine OPG [22-401] (U.S. Serial No. 08/577,788, filed December 22, 1995, now U.S. Patent No. 6,613,544), was digested with HindIII and NotI to remove the entire OPG reading frame. Murine OPG binding protein, residues 158-316 were PCR amplified using from the plasmid template pCDNA/32D-F3 using the following primers:

1616-44: CCT CTC TCG AGT GGA CAA CCC AGA AGC CTG AGG CCC AGC CAT  
TTG C

1602-59: CCT CTG CGG CCG CGT CTA TGT CCT GAA CTT TG